ene from 10 g. of chloro ketone was 1 g. melting at 173–174.5°. The synthesis was carried out in two ways.

(a) **From Phenylglyoxal and Phenacyl Bromide.**—One gram of phenylglyoxal was added rapidly, with vigorous stirring, to a mixture of 20 ml. of 10% sodium hydroxide solution, 15 ml. of dioxane and 3 g. of phenacyl bromide. After being stirred for fifteen minutes the mixture was shaken with ether. Evaporation of the ether gave a small quantity of solid which upon purification by recrystallization from ethanol, proved to be *sym*-dibenzoylethylene oxide.

(b) **From *sym*-Dibenzoylethylene.**—A mixture of *sym*-dibenzoylethylene, dioxane, 10% sodium hydroxide solution and sodium hypochlorite solution was shaken for a short period of time. An appreciable quantity of the oxide was removed by ether extraction.

Acetophenone and Sodium Hypoiodite.—A mixture of 20 g. of acetophenone, 30 ml. of dioxane and 200 ml. of 10% sodium hydroxide solution was stirred vigorously in the cold during the addition of 50 g. of iodine in aqueous potassium iodide solution. The addition was completed in one to two hours. The reaction mixture was extracted with benzene, and the residue obtained by distilling the benzene was freed from iodoform by steam distillation. From the residue by recrystallization from ethanol was obtained a small amount of a compound melting at 128–129°.[3]

Anal. Calcd. for $C_{16}H_{12}O_3$: C, 76.11; H, 4.76. Found: C, 76.0; H, 5.0.

It has been reported[5] that *sym*-dibenzoylethylene forms in 58–71% yields when phenacyl chloride is treated with aqueous potassium hydroxide. Attempts to use this method to make the mesityl analog, however, were unavailing. Treatment of α-chloroacetomesitylene in dioxane with 10% aqueous sodium hydroxide solution, alcoholic potassium hydroxide solution or sodium ethoxide failed to yield any *sym*-dimesitoylethylene.

(5) Bogoslov, *J. Gen. Chem. U. S. S. R.*, **14**, 993 (1944); *C. A.*, **39**, 600 (1945).

DEPARTMENT OF CHEMISTRY
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RECEIVED MARCH 30, 1946

Effect of Phenylacetic Acid Derivatives on the Types of Penicillin Produced by Penicillium Chrysogenum Q176

BY K. HIGUCHI, F. G. JARVIS, W. H. PETERSON AND M. J. JOHNSON

Because of recent emphasis on the differences in *in vivo* behavior among the various penicillins, data on the relative amounts of the penicillins produced by *P. chrysogenum* Q176 under various conditions become of interest. Some of our recent pertinent data are therefore summarized in Table I. The assay used to differentiate the penicillins is a microbiological procedure involving the use of four organisms, which will be published elsewhere. On known mixtures of three penicillins (G, X and K), the assay has given an average error of 10% in the estimation of the quantity of one component. Yields in the table are expressed as *S. aureus* units.

The following conclusions may be drawn from the table: (1) The penicillin produced in the absence of phenylacetic acid derivatives is largely K. Although the remainder has been calculated as G, its amount is too small to permit confidence in the result. (2) In the presence of corn steep liquor, which is known to contain phenylacetic acid derivatives, the proportion of G produced increases. The production of G is greatest early in the fermentation. (3) In the presence of added phenylacetic acid derivatives there is a great increase in the proportion of G. (4) The addition of a phenylacetic acid derivative always results in an increase in unit yield (and a larger increase in molar yield). (5) The addition of *p*-hydroxyphenylacetic acid results in the production of significant amounts of penicillin X, but phenylacetic

TABLE I

EFFECT OF PHENYLACETIC ACID DERIVATIVES ON PENICILLIN PRODUCTION

Run no.[a]	Compound added[c]	Age of fermentation, hr.	Total penicillin, u./ml.	Composition of penicillin, % G.	X[b]	K
1	None	42	94	13	...	87
		66	233	14	...	87
		75	264	12	...	88
	β-Phenylethylamine	42	117	91	...	9
		66	272	76	...	24
		90	435	66	...	34
	Phenylacetic acid[d]	42	169	88	...	12
		66	333	79	...	21
		75	387	74	...	26
	Phenylacetamide	42	119	91	...	9
		66	194	69	...	31
		90	384	57	...	43
2	None	60	161	44	3	53
		108	569	29	1	70
	β-Phenylethylamine	60	267	93	2	5
		108	726	78	5	17
	Phenylacetamide[d]	60	335	103	1	− 4
		108	616	78	2	20
	Phenylacetic acid[d]	60	448	109	3	−12
		108	673	76	−1	25
	p-Hydroxyphenylacetic acid	60	195	57	18	25
		108	422	42	11	47
	p-Hydroxyphenylacetic acid[d]	60	209	39	26	35
		108	462	34	10	56
3	None	49	638	30	...	70
4	Phenylacetic acid[e]	24	216	35	...	65
		60	1000	67	...	33
		72	1045	77	...	23

[a] Runs 1 and 2 were carried out in 500-ml. Erlenmeyer flasks containing 85 ml. (run 1) or 100 ml. (run 2) of medium. The medium used in run 1 consisted of the following constituents in grams per liter: lactose 22.5; glucose 7.5; ammonium lactate 7.1; potassium dihydrogen phosphate 2.0; magnesium sulfate 0.25; ferrous sulfate 0.20; copper sulfate 0.005; zinc sulfate 0.02; aluminum chloride 0.00027; potassium dichromate 0.000053. The medium used in run 2 contained the following constituents in grams per liter: corn steep liquor solids 30; lactose 30; calcium carbonate 10. Runs 3 and 4 were carried out in aerated and agitated tanks. The volume of medium used was 220 liters. The medium used in run 3 contained in grams per liter: steep liquor solids 40; lactose 40; calcium carbonate, 10; sodium sulfate 1.0. The medium used in run 4 was the same as that in run 3 except that the steep liquor concentration was 20 g. per liter. [b] When the differential assay results were calculated as a mixture of G and K only, no figure is given for X. [c] The compounds were added at a level of 0.5 g. per liter, and were added before sterilization unless otherwise stated. [d] The compound was added twenty-four hours after inoculation. [e] The phenylacetic acid was added in 6 equal portions, at 0, 12, 24, 36, 48 and 60 hours.

acid appears to be more available to the organism as a precursor than the hydroxy derivative.

DEPARTMENT OF BIOCHEMISTRY
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MADISON, WISCONSIN RECEIVED JUNE 13, 1946

The Molecular Size and Shape of Botulinus Toxin[1]

BY GERSON KEGELES[2]

Type A Botulinus toxin has been prepared in crystalline form by two independent groups of workers.[3,4] Concurrent with fractionation studies by these groups, the physicochemical properties of the toxin were investigated.[5] Electrophoretic investigations are reported elsewhere.[4] This communication reports conclusions as to the size and shape of the toxin molecule, based on studies of diffusion, apparent specific volume, and viscosity performed on crystalline materials.

The diffusion measurements were carried out in 0.06 molar sodium acetate buffer at *p*H 4.48 in the Tiselius electrophoresis apparatus[6] as described by Longsworth.[7] The results appear in Table I, where the diffusion constants have been averaged from both channels.

TABLE I

Da is calculated from height and area; *Dm* calculated by method of moments; $D_{20,w}$ calculated from average of *Da* and *Dm*.

Source	C-50 (Lamanna)[3]	XII (Abrams)[4]	XII (Abrams)[4]
Protein concn., %	0.47	0.98	0.50
Temp., °C.	1.0	1.0	20.0
$Da(10)^7$ cm.²/sec.	0.93	1.04	1.99
$Dm(10)^7$ cm.²/sec.	0.95	1.22	2.00
$D_{20,w}(10)^7$ cm.²/sec.	1.79	2.16	2.10

The lower value observed for the electrophoretically homogeneous fraction C-50 may be due, possibly, to partial denaturation resulting from the use of chloroform in its purification.

Density measurements on fractions prepared by both methods were made in an uncapped pycnometer at 30°. Since the protein becomes insoluble when dried, solutions for density determinations were prepared by dialysis against the buffers used as reference solvents. Protein concentrations in these solutions were determined by Kjeldahl nitrogen, using 14.2% as the best available figure for nitrogen content.[3,4] Apparent specific volumes in 0.06 molar sodium acetate buffer at *p*H 4.48 varied somewhat with concentration. On the assumption that the variation was a charge effect, the density measurement was repeated nearer to the isoelectric point[4] in the presence of a large excess of salt. The apparent specific volume obtained for a 0.59% solution of the salt-fractionated protein[4] in 0.2 molar sodium chloride–0.02 molar sodium acetate buffer at *p*H 5.38 was 0.76. The value of the partial specific volume corrected[8] to 20° is taken as $V_{20} = 0.75_5$.

Viscosity measurements at seven protein concentrations from 0.1 to 0.8 per cent. by weight in 0.06 molar sodium acetate buffer at *p*H 4.48 were made with an Ostwald viscometer, using fractions prepared by both methods. Although subsequent examination revealed extensive electrophoretic inhomogeneity in the crystalline chloroform-treated protein fraction studied,[3] satisfactory agreement in the viscosity data was obtained, giving an intrinsic viscosity of 10.6. This corresponds to an axial ratio of 8.3 according to the Simha theory[9] for elongated ellipsoids and a frictional ratio f/f_0 of 1.45 from the Perrin theory.[10] The isoelectric point[4] is 5.60 and further addition of neutral salt would suppress charge effects, giving a lower value for the frictional ratio.

The molecular weight *M* is 1,130,000 as calculated from the diffusion constant $D_{20,w} = 2.10(10)^{-7}$ cm²/sec., the partial specific volume $V_{20} = 0.75_5$ and the frictional ratio $f/f_0 = 1.45$ with the equation[11]

$$MV_{20} = [2.89(10)^{-5}/D_{20,w}(f/f_0)]^3$$

This must be regarded as a lower limit, because the error in the frictional ratio is tripled by this method of calculation. It is hoped that future ultracentrifuge studies which were not possible at the time of this investigation will improve the accuracy of the data. The large size of the molecule is particularly surprising in view of previous studies on bacterial toxins.[12]

(1) Investigations conducted at Camp Detrick, Frederick, Maryland, from July through October, 1945, by Gerson Kegeles, 1st Lt., CWS.

(2) Present address: Chemistry Department, University of Wisconsin, Madison, Wisconsin.

(3) Lamanna, McElroy and Ecklund, *Science*, **103**, 613 (1946).

(4) Abrams, Kegeles and Hottle, *J. Biol. Chem.*, **164**, 63 (1946).

(5) The author is indebted to the senior author of each group for the materials for these studies.

(6) Tiselius, *Trans. Faraday Soc.*, **33**, 524 (1937).

(7) Longsworth, *Ann. N. Y. Acad. Sci.*, **41**, 267 (1941).

(8) Svedberg and Pedersen, "The Ultracentrifuge," Oxford Press, 1940, Appendix II.

(9) Simha, *J. Phys. Chem.*, **44**, 25 (1940).

(10) Perrin, *J. phys. rad.* **VII**, **7**, 1 (1936).

(11) Reference 8, equation (70a).

(12) Krejci, Stock, Sanigar and Kraemer, *J. Biol. Chem.*, **142**, 737 (1942).

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FREDERICK, MARYLAND RECEIVED JUNE 8, 1946

Preparation of *p*-Alkylbenzyl Chlorides

BY G. M. KOSOLAPOFF

The preparation of *p*-alkylbenzyl chlorides has been effected usually by the method of Blanc[1] or by minor variations thereof. Such procedures utilize the catalytic effect of zinc chloride, which necessitates rather strict temperature control to avoid resinification and, generally, polysubstitu-

(1) Blanc, *Bull. soc. chim.*, (4) **33**, 313 (1923).